



Male acoustic display in the sand goby – Essential cue in female choice, but unaffected by supplemental feeding

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ABSTRACT

Many teleost fishes use acoustic and visual signalling during courtship. Such displays may convey information about body condition. Here we experimentally altered body condition of sand goby (*Pomatoschistus minutus*) males to examine effects on acoustic and visual courtship and subsequent spawning decisions. Over two weeks, males fed in excess were fed daily, whereas food-deprived males were fed once a week. Females only spawned with males that produced courtship sound. However, there were no treatment effects on the occurrence of spawning and males fed in excess did not invest more in visual or acoustic courtship than food-deprived males. That said, males fed in excess built more well-covered nests, with more sand piled on top, compared to food-deprived males. Male condition measured as lipid content differed significantly between treatments. However, only males fed in excess differed in lipid content from wild caught males, indicating that in nature, males are of similar condition to males in the low condition treatment group. Apart from the importance of courtship sound, the only male or female behaviour predicting reproductive success was if male displayed in the nest opening. Males often produce courtship sounds together with a visual display in this position. A female dark-eye display did not associate with reproductive success which, together with previous results, suggest a non-ornamental function of this trait. We conclude that male courtship sounds appear to be crucial in female mate choice, but the information content of the courtship sounds and how it relates to male condition remains elusive.

1. Introduction

Animals such as frogs, birds, mammals and insects produce sound during courtship and, like visual courtship, the acoustic courtship may carry information about nutritional condition (Scheuber et al., 2003), body size (Charlton et al., 2007), parasite load (Spencer et al., 2005), developmental stress (Spencer and MacDougall-Shackleton, 2011), 'good' genes (Welch et al., 1998) and other male quality traits. Teleost fish also produce sound in reproductive contexts, but the function of these vocalizations are not fully understood (Amorim et al., 2015). Aspects of the sound, such as spectral characteristics and sound pressure

level, correlate with body size, at least in some species (Lindström and Lugli, 2000; Malavasi et al., 2008; Pedroso et al., 2013; Verzijden et al., 2010). Accordingly, playback experiments have shown females to prefer males with certain aspects of the courtship sound including those associated with size (McKibben and Bass, 1998; Myrberg et al., 1986). In addition, Mann and Lobel (1995) observed that in the damselfish *Dascyllus albisella* the maximum male calling rate observed in the day prior to spawning was positively correlated with brood size. Male condition has been shown to correlate with aspects of acoustic courtship (Amorim et al., 2013b; Pedroso et al., 2013). The best example may be female Lusitanian toadfish (*Halobatrachus didactylus*) that prefer males with a

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higher vocal activity, which also reflects body condition (Amorim et al., 2010; Vasconcelos et al., 2012). In addition, courtship sounds vary between species and are potentially used in species recognition (Amorim et al., 2004; Blom et al., 2016; Crawford et al., 1997; Pedroso et al., 2013; Verzijden et al., 2010).

Gobies in the genus *Pomatoschistus* show male parental care in nests built by the male (Amorim et al., 2013b; Kvarnemo et al., 1998; Svensson et al., 2004). These species are well-studied regarding sound production and associated behaviours (Blom et al., 2016; Malavasi et al., 2012). Another research focus has been the condition (body fat reserves) of the male and its relevance in reproductive behaviours. Male condition has been shown to affect or be positively correlated with nest building (Kvarnemo et al., 1998; Lehtonen and Wong, 2009; Olsson et al., 2009), male nest defence (Svensson et al., 2004), mating success (Svensson and Forsgren, 2003) and hatching success (Kvarnemo et al., 1998). The well-covered nests associated with high condition appear to be preferred by females as spawning sites (Jones and Reynolds, 1999; Svensson and Kvarnemo, 2005). On the other hand, male visual display appears to be unaffected by manipulated condition (Olsson et al., 2009) though correlative studies have found it to correlate negatively (Amorim et al., 2013b; Svensson et al., 2004) with condition. Finally, natural variation in condition appears to affect acoustic display rate positively (Amorim et al., 2013b; Pedroso et al., 2013). Accordingly, previous studies have shown that females prefer (Amorim et al., 2013a) and only spawn with males that produce courtship sounds (Amorim et al., 2013b), and seem to prefer males with a higher drumming rate (Amorim et al., 2013b; Pedroso et al., 2013).

In this study, we manipulated body condition of sand goby, *Pomatoschistus minutus* (Pallas), males by feeding regime, to examine whether body condition affects nest building, visual courtship and acoustic courtship and if it influences female mating decision in a single male 'no choice' setup. Based on previously published results reviewed above we predicted that males fed in excess would invest more than food-deprived males in 1) nest building and 2) acoustic courtship and 3) achieve higher mating success. We also predicted that 4) visual courtship would be unaffected by treatment or that food-deprived males would display more than males fed in excess. In addition, we analysed how nest characteristics and courtship behaviours predicted the occurrence of dark eyes in females, a trait displayed by ready to mate females (Blom et al., 2016; Olsson et al., 2017; Olsson et al., 2022), and spawning success.

2. Materials and methods

2.1. Study species

The sand goby is a small (max length 9.5 cm TL), marine fish distributed in lagoons, coastal areas and estuaries of the NE Atlantic, Mediterranean and Baltic region (Kullander et al., 2012; Miller, 1986). These short-lived fish have a single breeding season between March and August (Kullander et al., 2012), during which both sexes can reproduce sequentially with different mates. Males develop a nuptial colouration with black pigmentation on pelvic, anal and tail fins. The anal fin has a clear blue colouration with a black trim, and there is a eye-spot on the first dorsal fin. This colouration is lacking in the cryptic female (Forsgren, 1999; Kullander et al., 2012). Males build a nest by covering bivalve shells with sand and compete for mating opportunities with females, which in turn are the choosier sex. Male courtship behaviour normally starts with a visual display in which the male approaches the female with erect ornamented fins. Typically, the male swims back to the nest in a conspicuous manner, considered a lead display ('lead swim'), and the female may choose to follow the courting male to his nest (reviewed in Blom et al., 2016; Forsgren, 1999). The male might produce a series of sounds both when the female is outside the nest and when she has entered it (Blom et al., 2016; Lindström and Lugli, 2000; Pedroso et al., 2013). In the sand goby, the sound consists of trains of pulses (drums) which typically are grouped in bursts with <0.3 s

interval between drums (Lindström and Lugli, 2000; Pedroso et al., 2013). During courtship and before entering the nest, the females sometimes get conspicuous dark eyes (Blom et al., 2016; Olsson et al., 2017; Olsson et al., 2022). After entering the nest, females may still choose not to spawn and leave (Lehtonen and Kvarnemo, 2015). The video clip in the supplementary material include male visual and acoustic courtship as well as female dark eyes.

2.2. Experimental design

The experiment was conducted at Kristineberg Marine Research Station on the west coast of Sweden (58°15' N, 11°27' E) between May and July 2013–2014. All fish were caught by hand trawling in bays nearby the station. Males were randomly assigned to one of two treatments. During treatment, males were kept in same sex groups in four 50-l aquaria. In a food excess treatment males were fed every day for two weeks and in a food-deprived treatment males were fed twice during the same period (on day 1 and day 7). In the food excess treatment, the uneaten food was removed once a day and replaced with new food. The food-deprived males had not been fed for a week when entering the experiment. These males were thereafter either used in the behavioural experiment or analysed for lipid content (see below). Females were housed in same sex 50 l storage aquaria and fed daily for 7 days before the experiment started. All fish were fed commercial fish food granules (Nutra HP, Skretting). Any uneaten food was removed the day after feeding. All aquaria had a continuous flow of natural seawater (salinity 22–31 PSU, 11–12 °C) and natural daylight was provided through windows.

In the behavioural experiment, we used 20 experimental glass aquaria (dimensions (LxWxH): 30 cm × 25 cm × 30 cm) filled to 20 l. Each aquarium contained a 5-cm layer of sifted sand and was equipped with an artificial nest site, made of a half grey polypropylene tube (Ø 56 mm) with a pipe attached to a hole, like a chimney (Ø 20 mm). A hydrophone was later placed in the chimney. The video clip in the supplementary material shows the experimental set-up. Each nest site was fitted with an acetate sheet lining the ceiling for females to lay eggs on. The experimental aquaria were placed on top of two marble layers interspaced with silicon balls for sound insulation from ground borne vibrations. The aquaria were separated by opaque screens to avoid visual contact between fish in adjoining replicates.

Males were placed individually in an experimental aquarium in the evening. To encourage nest building, a ripe female in a perforated transparent vial was placed in front of the nest site and the nest was photographed 12 h later. Thereafter, the female was removed and replaced with two new ripe females. These new females were left in a vial inside the aquarium for 1 h to acclimatise before they were released in the aquarium.

Behavioural interactions and courtship sounds were recorded in the first 60 min after the females were released, using a camcorder (Canon Legria HF M56, Ōta, Tokyo, Japan) placed in front of the aquarium at a 90 cm distance. Courtship sounds were registered using a calibrated hydrophone (HTI-96-MIN with pre-amplifier, High Tech Inc., Gulfport MS; sensitivity: −165 dB re. 1 V/μPa, frequency range: 0.02–30 kHz) connected to a digital audio recorder (Song Meter SM2+, Wildlife Acoustics, Inc., Massachusetts, USA, sampling frequency 16 kHz 2013 and 32 kHz 2014).

The replicates were terminated 12 h after the females were released. At this point, the fish were removed from the aquaria and the nests were examined for eggs. All fish used in the experiment were fed and returned to their natural environment. We successfully filmed 15 males from the food excess treatment and 16 males from the food-deprived treatment.

2.3. Lipid analysis

To measure the effect of the feeding treatment on lipid reserves, we extracted the lipid contents from 11 males fed in excess, 12 food-

deprived males and 12 wild-caught males, following a protocol similar to previous studies (e.g. Lehtonen and Wong, 2009; Olsson et al., 2009; Pedroso et al., 2013). These males were not used in the behavioural experiment but were, before euthanasia, treated identically. The males were euthanized using MS222 and frozen individually in -20°C . The food excess males and the food-deprived males had not been fed for 24 h and 7 days, respectively, whereas the wild-caught males were euthanized immediately upon capture. Defrosted fish were dried in a desiccation oven at 72°C for 36 h before being weighed to the nearest 0.001 mg using a microbalance (model XR 205SM-DR, Precisa Instruments Inc., Switzerland). All further steps using petroleum ether were performed in a fume cupboard. To extract the lipids the whole fish was put in a glass vial and submerged in petroleum ether for 12 h. After removing the fish from the vial, it was left for at least 2 h in the fume cupboard for excess petroleum ether to evaporate. The fish were then dried again overnight at 72°C in the desiccation oven before being weighed a second time. The difference in body mass between the first and second weighing estimates the lipid content of the fish.

2.4. Data collection

Nest building effort was measured as the volume of sand piled on top of the nest and nest opening width. Nest volume was measured from the photographs of the nests, and visually compared to a series of photographs of nests with known volumes of sand (Svensson and Kvarnemo, 2005). Nest opening width was measured from the same photographs using the program ImageJ 1.51 (<https://imagej.net>) with the diameter of the chimney used as a scale. Visual courtship of males (the total time period displaying outside and inside the nest) and the occurrence of dark eyes in females were measured from the videos for the whole 60 min using an event recorder (JWatcher + video 1.0; <https://www.jwatcher.ucla.edu>) (see Blom et al. (2016) for a description of the behaviours). The observer was blind to treatment. All males were monitored for acoustic displays. Out of the 31 filmed males, 17 males produced sound. However, four sound files did not have good enough quality for sound analysis due to high background noise levels and we also lost two sound files due to an accidentally reformatted hard drive. Therefore, 6 well fed and 5 food-deprived males were analysed regarding number of and duration of sounds (drums and bursts) and sound pressure level (SPL), from which 5 well fed and 5 food-deprived males were analysed for number of pulses per drum (train) and pulse repetition rate (number of pulses per second in the drum). Calling effort, i.e. the proportion of the time spent vocalising was tallied for the total time period. Active calling rate (number of drums per minute) was measured during the periods when the males produced sounds. No burst of drums continued for longer than one minute and we counted the number of drums during one minute starting with first drum of the drum burst. We used the average for all drum bursts in the 1 h recording. Duration of sound bursts (a sequence of drums) was the average of the first three bursts (3 being the minimum of bursts per male). For the analysis of pulse rate (number of pulses per second), we used the average of the first three successful analyses in the sequence of drums. Relative SPL was based on the average of three drums produced when the males were in the same position relative to the hydrophone i.e., head in the entrance of the nest and the body inside, while the female was outside the nest. We used the Aquatic acoustic metrics interface (AAMI) software to calculate the maximum (SPL_{max}) and the average (root mean square, SPL_{rms}) sound pressure levels (dB re $1 \mu\text{Pa}$). For the other sound analyses, we used Matlab R2009b (The Mathworks Inc., Natick, Massachusetts, USA).

2.5. Statistical analysis

Treatment effects on continuous response variables (except proportions) were analysed with generalized linear models with linear scale response, scale parameter method set to maximum likelihood estimate and model-based estimators. Treatment effect on lipid content of males

was analysed with lipid-free dry body mass as a covariate. We used sequential Bonferroni corrections in the post hoc test on lipid reserves. Proportions (time males spent on courtship) were analysed with a binary logistic response, with the event courtship and vocalization being the event occurring during the observation time, scale parameter set to deviance and model-based estimators. For courtship, observation time was the total time when the female was outside of the nest, for calling effort (all males) the whole hour and for calling effort (calling males) the time between the start of the first call and the end of the last call. The effect of treatment on the occurrences of courtship sound, female dark eyes and spawning was analysed with a $2 \times 2 \chi^2$ -test.

When correlating the occurrence of spawning and female dark eyes we used ordinal logistic models with scale parameter method set to fixed value and model-based estimator.

In the analyses of lipid data, three outliers had to be removed to avoid spurious interactions. These are marked with Δ for a food-deprived male and + for wild caught males in Fig. 2. Apart from the interaction, the results were qualitatively the same when the outliers were included. Three nests were affected by the water inlet and were therefore not analysed regarding nest cover and nest opening. As mentioned above, not all males producing sound were analysed, due to background noise on the sound files and a reformatted hard drive. All tests have $df = 1$ (two tested groups) except the lipid analysis which has $df = 2$ (three tested groups). We report estimated marginal mean \pm standard error of mean (SE) when given by the models, otherwise we report the true mean \pm SE.

All statistical analyses were performed in SPSS (IBM SPSS Statistics for Windows, Version 27.0).

2.6. Ethics

We followed the Guidelines for the treatment of animals in behavioural research and teaching published in each January issue of Animal Behaviour. This study complies with the Swedish law. The Ethical Committee for Animal Research in Gothenburg granted our permits for the animal experiments (permit numbers: 135–2010 and 143–2012). All fish were released into their natural environment or euthanized with an overdose (50–100 mg/l) of MS222 for at least 30 min ensuring the destruction of the brains.

3. Results

3.1. Effect of food regime on nest-building effort, courtship and mating success

Nest cover differed significantly between treatments, with males fed in excess building more well-covered nests. Likewise, nest openings differed in width, with males fed in excess building smaller openings, although this difference was not significant. There were no significant treatment effects on the time the male spent on visual courtship, the occurrence of female dark eyes, or on male mating success (Table 1). Furthermore, there were no significant treatment effects on the occurrence of acoustic courtship or on any of its properties including active calling rate (Fig. 1, Table 2).

3.2. Effect of acoustic and visual displays on female dark eyes

Females were not more likely to display dark eyes in response to visual courtship or in replicates where the male produced courtship sound. However, nest-building effort, measured as nest cover and nest opening width, was higher in replicates where females showed dark eyes than in those where females did not. For nest cover this was significant, whereas for nest opening size it was a non-significant trend (Table 3).

Table 1
Treatment effects on nest building, visual courtship and spawning success.

Endpoint	Food excess (estimated marginal mean \pm SE or n)	Food-deprived (estimated marginal mean \pm SE or n)	χ^2	n (excess: deprived)	p
Nest cover (cm ³)	337 \pm 23	268 \pm 24	4.12	15:13	0.042
Nest opening width (mm)	17.7 \pm 3.0	25.8 \pm 3.2	3.49	15:13	0.062
Courtship outside nest (proportion)	0.310 \pm 0.054	0.274 \pm 0.052	0.219	15:16	0.64
Courtship inside nest (proportion)	0.145 \pm 0.041	0.110 \pm 0.037	0.398	15:16	0.53
Female dark eyes (yes: no)	7:8	7:9	0.027	15:16	0.87
Spawning (yes:no)	6:9	7:9	0.045	15:16	0.83

Nest cover is the amount of sand piled on top of the nests. Proportional courtship was calculated for the observations when no females were in the nests. Means were tested with generalized linear models and Wald statistics and counts with χ^2 -tests.

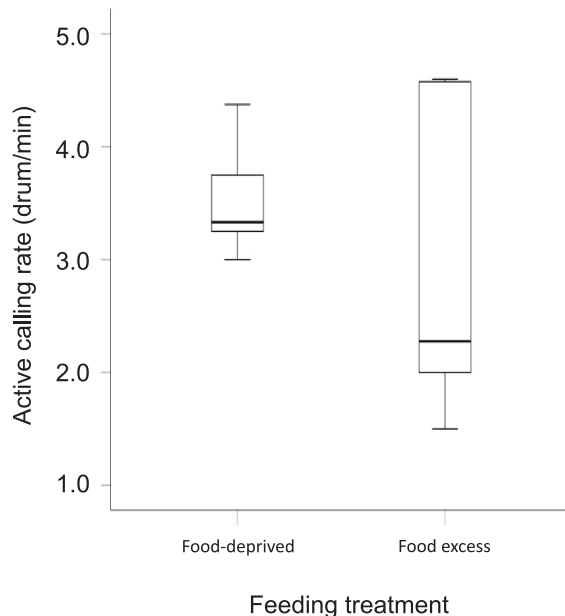


Fig. 1. Active calling rate (drum per minute) in sand goby males (*Pomatoschistus minutus*) of males from two different feeding treatments. Active calling rate is the average number of drums per minute considering only the minutes when the fish were vocally active. The average for all sound periods was used. Active calling rate has previously been shown to correlate positively with the relative lipid content of the males (Pedroso et al., 2013). The boxplots show medians, 25th and 75th quartiles, and minimums and maximums.

3.3. Effect of acoustic and visual displays on male mating success

Nest cover, nest opening width or visual courtship outside the nest by the male, or dark eyes being displayed by the female did not predict spawning. However, both visual courtship by the male inside the nest and the occurrence of courtship sound correlated positively with spawning. In fact, spawning only happened in replicates where the male produced courtship sound (Table 4).

3.4. Effect of food regime on male lipid content

There was a significant effect of feeding treatment on lipid content of the males (generalized linear model: treatment: Wald $\chi^2 = 708.32$, $df = 2$, $p < 0.001$, dry weight: Wald $\chi^2 = 61.62$, $df = 1$, $p < 0.001$). In the post hoc tests, food excess males had significantly more lipids than food-deprived and wild caught males (both $p < 0.001$) whereas food-deprived males did not differ significantly from wild caught males ($p = 0.095$) (Fig. 2).

Table 2
Treatment effects on courtship sound.

Male acoustic courtship measure	Food excess (estimated marginal mean \pm SE or n)	Food-deprived (estimated marginal mean \pm SE or n)	χ^2	n (excess: deprived)	p
Courtship sound (yes: no)	10:5	7:9	1.64	15:16	0.20
Calling effort (all males, proportion)	0.0076 \pm 0.003	0.0043 \pm 0.002	0.68	11:14	0.41
Calling effort (calling males, proportion)	0.024 \pm 0.07	0.039 \pm 0.013	0.87	6:5	0.35
Active calling rate (drums min ⁻¹)	2.9 \pm 0.4	3.5 \pm 0.4	1.28	6:5	0.26
Burst duration (s)	3.2 \pm 0.6	4.5 \pm 0.6	2.34	6:5	0.13
Number of pulses per drum	70.6 \pm 7.3	72.7 \pm 7.3	0.04	5:5	0.84
Pulse repetition rate (pulse/s)	26.9 \pm 2.4	25.6 \pm 2.4	0.15	5:5	0.70
SPL _{rms} (dB re 1 μ Pa)	92.2 \pm 2.1	94.5 \pm 2.3	0.55	6:5	0.46

Courtship sound is the incidence of male courtship sound. Calling effort (all males) is the proportion spent calling during one hour whereas calling effort (calling males) is the proportion spent calling from the start of the first call until the end of the last call. Active calling rate is the average number of drums per minute considering only the minutes when the fish were vocally active. The average for all sound periods was used. Relative sound pressure levels were measured when the male had the head in the entrance (root mean square, SPL_{rms}). The averages of three measures were used in the analyses. Means were tested with generalized linear models and Wald statistics and counts with χ^2 -tests.

4. Discussion

In the present study, sand goby females only spawned with males performing acoustic courtship. We thereby confirm previous findings from the same genus that female *P. minutus* and *P. pictus* do not spawn with silent males (Amorim et al., 2013b; Lindström and Lugli, 2000; Pedroso et al., 2013). Of 15 males fed in excess, 10 males (67%) produced sound whereas 7 of 16 food deprived males (44%) did so. However, this difference was not significant and male spawning success was independent of feeding treatment. Our food excess treatment affected the energy reserves of the fish as intended, such that the lipid content of males fed in excess was significantly higher than that of food-deprived males. However, food-deprived males were of similar condition as wild caught males, which limits the interpretations of our results. Food-deprived males performed acoustic courtship as often as males fed in

Table 3
Behaviours associated with female dark eyes.

Measured male behaviour	Dark eyes (mean ± SE or n)	No dark eyes (mean ± SE or n)	χ^2	n (dark: no dark)	p
Nest cover (cm ³)	350 ± 20	271 ± 25	3.834	14:14	0.050
Nest opening width (mm)	16.0 ± 2.6	25.4 ± 3.3	3.332	14:14	0.068
Visual courtship outside nest (proportion)	0.320 ± 0.043	0.279 ± 0.051	0.377	14:17	0.54
Visual courtship inside nest (proportion)	0.127 ± 0.027	0.132 ± 0.045	0.046	14:17	0.83
Courtship sound (yes: no)	9:5	8:9	0.920	14:17	0.34

Nest cover is the amount of sand piled on top of the nests. Proportional courtship was calculated for the observations when no females were in the nests. Courtship sound is the incidence of courtship sound. Means were tested with generalized linear models and Wald statistics and counts with χ^2 -tests.

Table 4
Behaviours associated with spawning success.

Measured male behaviour	Spawning (mean ± SE or n)	No spawning (mean ± SE or n)	χ^2	n (spawning: no spawning)	p
Nest cover (cm ³)	315 ± 24	297 ± 27	0.274	13:15	0.60
Nest opening width (mm)	19.9 ± 3.7	22.7 ± 3.1	0.369	13:15	0.54
Visual courtship outside nest (proportion)	0.294 ± 0.033	0.299 ± 0.053	0.005	13:18	0.94
Visual courtship inside nest (proportion)	0.198 ± 0.124	0.077 ± 0.035	3.873	13:18	0.049
Courtship sound (yes: no)	13:0	4:14	18.44	13:18	<0.001
Female dark eyes (yes: no)	6:7	8:10	0.009	13:18	0.95

Nest cover is the amount of sand piled on top of the nests. Proportional courtship was calculated for the observations when no females were in the nests. Courtship sound is the incidence of courtship sound. Means were tested with generalized linear models and Wald statistics and counts with χ^2 -tests.

excess once a day. Furthermore, no measures of courtship sound or visual courtship suggested any increased investment in courtship among males fed in excess. Still, males fed in excess piled more sand on top of the nest, and tended to have smaller nest openings, but nest-building effort did not affect spawning success.

The finding that males fed in excess did not increase their acoustic signalling effort compared to food-deprived males is in contrast with previous correlative studies. In the same sand goby population as used in the present study, natural variation in body lipid content has been shown to correlate positively with the active calling rate (number of drums per minute) (Pedroso et al., 2013). The same correlation was found in the congener painted goby as well as a correlation between calling effort (time proportion producing sound) and male body condition (Amorim et al., 2013b). There were no effects of feeding treatment on corresponding measures of calling effort and active calling rate in the present study. In a similar manner, manipulated fat-reserves have been shown to have no effect on male visual display rate (Olsson et al., 2009) whereas natural variation in fat-reserves correlated negatively with male display (Svensson et al., 2004). The results from the measures of

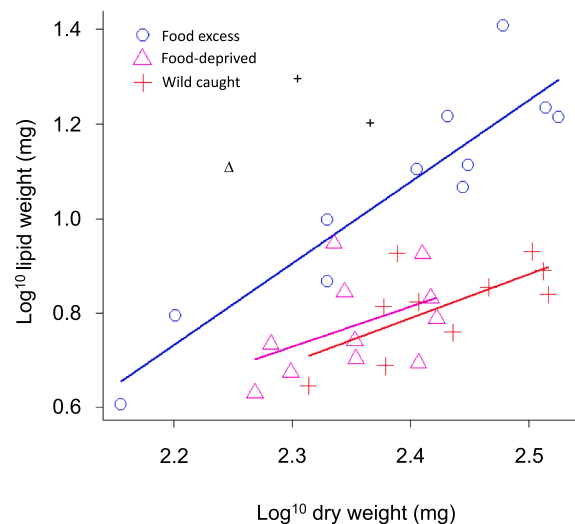


Fig. 2. Lipid content in relation to dry weight in sand goby males (*Pomatoschistus minutus*). Food excess males were fed every day during two weeks whereas food-deprived males were fed twice in the same period. Wild caught males were not fed and euthanized immediately after capture. Food excess males differed significantly in lipid content in relation to their body mass from the two other groups of males, whereas there was no significant difference between food-deprived and wild caught males. Three outliers had to be removed to avoid spurious interactions. These are marked with small Δ (food-deprived male) and small + (wild caught males). Note that the food excess and food-deprived males were from the same feeding treatments as the males in the behavioural analyses, however, their behaviours were not measured.

the sound produced should be treated with caution because sample size is low and they differ slightly from previous measures (Amorim et al., 2013b; Pedroso et al., 2013). However, one explanation for the lack of effect in the current study may be that the food deprivation was not severe enough to affect the males' acoustic and visual courtship. Indeed, wild-caught males had similar lipid content as food-deprived males. This shows that our restricted diet resulted in fish with energy reserves that correspond to natural levels, whereas the excess diet resulted in unnaturally fat fish. Furthermore, in the lipid analyses, a few males deviated from the expected positive relationship between lipid content and lean body mass, and were therefore treated as outliers. If they indeed are part of the natural variation a few individuals of the wild population may be of as high condition as the food-excess males of the present study. Alternatively, in natural populations, it is possible that lipid content, display rate and calling rate co-vary with other factors and that the information content of the courtship sound is not condition per se but instead reflects other aspects of male quality including 'good' genes. In addition, previous studies have indicated that, at least without competition, males in low condition may increase their signalling effort (Candolin, 2000; Svensson et al., 2004). When other males are present, male signalling become more honest (Candolin, 2000; Gavassa et al., 2012). Indeed, one difference between the present study and Pedroso et al. (2013) is that in the latter, males had both visual and acoustic access to other nesting males. Furthermore, males may strategically focus their signalling effort on certain traits or aspects of the trait (Amcoff et al., 2009; Rebar and Rodriguez, 2016; Svensson and Kvarnemo, 2005). Therefore, future studies are needed to further investigate information content of the courtship sounds and the relation between male condition and acoustic behaviours.

Previous work on the genus *Pomatoschistus* in both aquarium experiments (Kvarnemo et al., 1998; Olsson et al., 2009) and in the field (Lehtonen and Wong, 2009) have shown that well-fed males and males with high lipid content pile more sand on top of their nests than other males, and that females prefer to spawn in more well-built nests (Jones and Reynolds, 1999; Svensson and Kvarnemo, 2005). It makes sense that

females chose well-covered nests to avoid spawning with males in bad condition to reduce the risk of filial cannibalism (Knapp and Kovach, 1991; Kvarnemo et al., 1998; Neff, 2003). However, nest coverage did not affect spawning decisions in the present study with its 'no choice' set-up. Apart from males performing acoustic courtship or not, the only behaviour predicting spawning was that the male courted visually inside the nest, a courtship that often includes acoustic courtship (Amorim et al., 2013b; Pedroso et al., 2013). Therefore, it appears that sand goby females may pay attention to nest cover early in the mate choice process, or if offered multiple males to choose between, but when it comes to the actual spawning decision, the courtship sound and maybe other aspects of within nest courtship are more important. Females of the sand goby temporarily show dark eyes before they spawn. Although rounder females are more likely to show this display, it does not appear to be a signal involved in mate choice by the male or directed to other females (Olsson et al., 2017). In line with those results, female dark eyes did not predict spawnings in the present study. That said, nests in replicates where females showed dark eyes were better covered.

To conclude, we found that experimentally increased body condition had no effect on visual and acoustic display or mating success. Yet, the acoustic courtship of sand goby males appears to be essential for female spawning decisions. However, the information content of the courtship sounds, and how it relates to male condition and other male traits remains elusive.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jembe.2022.151791>.

Data availability statement

The data is available in the supplement.

CRedit authorship contribution statement

Eva-Lotta Blom: Methodology, Formal analysis, Investigation, Writing – original draft, Funding acquisition. **Joanna Wilson:** Methodology, Investigation. **Charlotta Kvarnemo:** Resources, Writing – review & editing, Supervision, Funding acquisition. **M. Clara P. Amorim:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Ola Svensson:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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